REMARKS / ARGUMENTS

Support for Amendments

The amendments are supported throughout the specification and drawings as originally filed. The following provides specific reference to passages in support of each amendment.

Claim 1 is amended to incorporate the elements of claim 138. Accordingly, Applicants have canceled claim 138. More specifically claim 1 is amended to recite that the at least two electrodes are disposed on the surface of the membrane.

Claim 1 is also amended to recite, "the migration of cells through the porous membrane permits contact between the migrating cells and one or more of at least two electrodes of said lower chamber." Support may be found in paragraph [0016] which recites in part, "Migration of cells through the porous membrane permits contact between the migrating cells and one or more electrodes of the lower chamber."

Claim 1 is also amended to recite, "wherein said at lest two electrodes have substantially the same surface area." Support may be found in paragraph [0087], which recites in part,

> "As used herein, "the at least two electrodes (electrode arrays) have substantially same surface area" means that the surface areas of any electrodes (electrode arrays) are not substantially different from each other so that the impedance change due to cell attachment or growth on the larger electrode (electrode array) will contribute to the overall detectable change in impedance to a same or similar degree as the impedance change due to cell attachment or growth on the smaller electrodes (electrode arrays)."

Further support may be found in Figure 1, 8, 9, 12, 15, 17 and 19 where the two electrodes for monitoring cell migration/invasion (for a single top/bottom chamber unit) have substantially same surface areas. Additional support may be found in paragraph [00109], which recites in part,

> "...the electrodes between or among which the impedance is measured to monitor cell migration ... can have substantially same ... surface area".

Claim 1 is also amended to recite, "contact at any one or more of said at least two electrodes provides a detectable change in impedance between or among the electrodes." Support may be found in paragraph [0016], which recites in part,

> "Migration of cell through the pores membrane permits contact between the migrating cells and one or more electrodes of the lower chamber. The contact provides a detectable change in impedance between or among the electrodes."

Claim 8 is amended to recite the thickness of membrane is from 5 microns to 50 microns. Support may be found in paragraph [00101], which recites in part, "Preferably, a biocompatible membrane of a device of the present invention is between about 5 and about 50 microns thick[.]".

Claim 24 is amended to recite the biocompatible membrane is flexible. Support may be found in paragraph [00120], which recites in part, "Due to the "flexible" nature of thin membrane, special care may need to be taken in order to reproducibly and accurately make microelectrode structures on the membrane using photolithography methods."

Claim 24 is also amended to recite the two or more electrodes fabricated on one side of a flexible biocompatible membrane have "substantially the same surface area." Support may be found in paragraph [0087] which recites in part,

> "As used herein, "the at least two electrodes (electrode arrays) have substantially same surface area" means that the surface areas of any electrodes (electrode arrays) are not substantially different from each other so that the impedance change due to cell attachment or growth on the larger electrode (electrode array) will contribute to the overall detectable change in impedance to a same or similar degree as the impedance change due to cell attachment or growth on the smaller electrodes (electrode arrays)."

Support may further be found in paragraph [00110], which recites in part,

> "In preferred aspects of the present invention, two or more electrodes are fabricated on one side of a biocompatible membrane. Preferably at least two of the two or more electrodes have substantially the same area."

Claim 24 is also amended to newly recite, "cell attachment or growth results in cellular contact with at least one of said two or more electrodes further resulting in a detectable change in electrical impedance, resistance or capacitance." Support may be found in paragraph [0090], which recites in part,

> "As used herein, "detectable change in impedance between or among the electrodes" means that the impedance between or among the electrodes would have a significant change that can be detected by an impedance analyzer or impedance measurement circuit when cells attach or grow on surfaces of the apparatus.... In addition, impedance has two components, resistance and reactance (reactance can be divided into two categories, capacitive reactance and inductive reactance)."

Further support may be found in paragraph [0041] describing Figure 14, which recites in part,

> "... Electrode arrays are on the bottom surface of the tarns-well membrane 20 ... The impedance change of the electrodes as a result of adhesion of cells that have migrated from the top chamber reflects the number of the cells migrated from the top chamber")."

Additional support may be found in paragraph [00113], which recites in part,

> "Electrodes or electrode elements are preferably distributed over the entire surface region of the device they are fabricated on, wherein such surface region is or will be exposed to contact by cells."

Additional support may be found in paragraph [00171], which recites in part,

> "... the attachment or detachment of one or more cells on the side of the membrane on which said at least two electrodes are fabricated can be detected by a change in impedance, capacitance, or resistance using the device."

Claim 25 is amended to recite the thickness of said membrane is from 5 microns to 50 microns. Support may be found in paragraph [00101], which recites in part, "Preferably, a biocompatible membrane of a device of the present invention is between about 5 and about 50 microns thick[.]"

Claim 51 is amended to recite that for each of the plurality of isolated fluid containers that comprises a single IDES or CCES, the exposed surface area of the one side of the biocompatible membrane on which electrodes are fabricated comprises an approximately uniform distribution of electrodes or electrode elements. Support may be found in paragraph [00143], which recites,

> "The device having two or more IDESs or CCESs can be preferably be part of an apparatus in which the device is reversibly or irreversibly attached to one or more structures that provide a plurality of isolated fluid containers each of which can comprise one or more IDESs or CCESs, such that the biocompatible membrane separates the fluid containers into upper chambers and lower chambers."

Claims 139, 140 and 141 are amended to depend from a pending claim.

Claim 145 is amended to recite the two or more electrodes are on the upper side of the membrane. Support may be found in paragraph [00147], which recites in part, "In some aspects of the present invention, the electrodes are fabricated on the upper side of the membrane."

Claim 146 is amended to recite the elements of claim 50.

Claim 148 is amended to correct dependency.

Claim 154 is newly added and provides the at least one pore of the biocompatible membrane has a diameter of less than 5 microns. Support may be found in paragraph [00107], which recites in part, "For example, the one or more pores can be less than about 5 microns, or preferably less than about 1 micron."

Claim 155 is newly added and provides the membrane includes a layer of cells on the upper side of the membrane, wherein the calls are Caco-2 cells. Support may be found in paragraph [00152], which recites in part,

> "The apparatuses of the present invention can be used for evaluating the integrity of the cell monolayer (Caco-2 cell

> layer). For such an application, the electrodes are fabricated on the upper side of the membrane...Similar to conventional Caco-2 assays, Caco-2 cells are then cultured on the membrane in the wells contained in the insert plate."

Claim 156 is newly added and provides a method for measuring the integrity of a cell monolayer, which includes providing the apparatus of claim 145; culturing cells in the upper chamber of the apparatus and monitoring the integrity of the cell monolayer in the upper chamber by monitoring the impedance. Support may be found in paragraph [00152], which recites in part,

> "The apparatuses of the present invention can be used for evaluating the integrity of the cell monolayer (Caco-2 cell layer). For such an application, the electrodes are fabricated on the upper side of the membrane...Cell monolayer integrity can be monitored by measuring the impedance between the electrodes fabricated on the membrane. The higher the electrical impedance, the more confluent or more compact or more tight the cell monolayer is."

Response to Claim Objections

Claims 146 and 148-150 were objected to under 37 C.F.R. 1.75(c) as being improper form because a multiple dependent claim should refer to other claims in the alternative only.

Claims 146 and 148 have been amended to remove multiple dependency. Claims 149 and 150 depend from amended claim 148.

Follow up to Recent Examiner Interview and Response to Examiner Summary

Applicants graciously thank the Examiner for the interview on November 8, 2007. In the Examiner interview on November 8, 2007 and in the follow up Examiner interview summary mailed November 14, 2007, the Examiner noted that comments with respect to the position of the electrodes on the membrane appeared to be persuasive in view of the prior art rejection of record. At the time of the interview, claim 24 included this limitation however claim 1 did not. Applicants have amended claim 1 to newly recite

that at least two electrodes are disposed on the porous membrane. For completeness, Applicants address the rejections of record.

Response to Claim Rejections Under 35 USC §103(a)

A. Claims 1, 2, 6, 8-11, 24, 25, 28, 29, 49, 138, 143-145, 147 and 151-153 are Not Obvious Over Picard (US 2004/0091397) or Tchao (US 5,601,997) in View of Lynes (US 6,723,523)

The Examiner has rejected Claims 1, 2, 6, 8-11, 24, 25, 28, 29, 49, 138, 143-145, 147 and 151-153 under 35 U.S.C. §103(a) as allegedly being obvious over Picard or Tchao in view of Lynes. More specifically, the Examiner alleges both Picard and Tchao disclose devices for monitoring the migration or invasion of biological particles. The Examiner argues that Picard discloses an upper chamber (114); a lower chamber (116); a biocompatible porous membrane (106) having a porosity sufficient to allow cells to migrate there through and the membrane (106) separates the upper and lower chambers (see Fig. 1 B). Similarly, the Examiner argues Tchao discloses an upper chamber (24); a lower chamber (22, 28); a biocompatible porous membrane (10) having a porosity sufficient to allow cells to migrate there through and the membrane separates the upper and lower chambers (See Fig. 2).

With respect to claim 1, the Examiner argues while both of the references of Picard and Tchao disclose the use of optical detection devices (see sensor 120 of Picard and detector 30 of Tachao) for detecting the presence of cells within the lower chamber, the references do not disclose that at least two electrodes are present in the lower chamber for detecting the presence of cells by a change in impedance between electrodes. However, the Examiner argues Lynes discloses that it is known in the art to employ impedance sensing electrodes within a cell or culture space for detecting the presence and/or movement of a cell in response to a chemotactic gradient (See col. 9, ll. 55-67).

Therefore the Examiner argues that it would have been obvious to one of ordinary skill in the art to replace the optical detection systems of the primary references with an impedance measurement system suggested by the reference of Lynes for the known and

expected result of providing an alternative means recognized in the art to detect or sense the presence of cells within the lower chambers of the test devices.

In an Examiner interview on October 22, 2007 and in the corresponding interview summary mailed October 31, 2007, the Examiner indicated that if the limitation set forth in claim 36 (namely where the at least two or more electrodes have substantially the same surface area) was incorporated into claim 1 or 24 then arguments should also address the references cited against claim 36. Thus in addition to the response to rejections as provided in the office action, Applicants' will also address the references provided against claim 36. Arguments against claim 1 are provided in section 1 below and arguments against claim 24 are provided in section 2C.

Applicants' Response

1. Claims 1, 2, 6, 8-11 and 138

In response to Applicants' arguments previously filed on 6/21/2007, the Examiner has noted that, "In response to applicants' argument that the references fail to show certain features of applicants' invention, it is noted that the features upon which applicant relies (i.e., all the electrodes of the device can be used to monitor impedance) are not recited in the rejected claim(s).

Applicants have canceled claims 2 and 138 and amended claim 1, from which claims 6, and 8-11 depend, to recite that at least two electrodes are disposed on the porous membrane and that the porous membrane of Applicants' invention permits contact between the migrating cells and one or more of at least two electrodes of the lower chamber, wherein the at least two electrodes have substantially the same surface area and further wherein the contact at any one or more of the at least two electrodes provides a detectable change in impedance between or among the electrodes. Thus Applicants have included the recitations that at least two electrodes are disposed on the porous membrane and the at least two electrodes have substantially the same surface area and contact at any one or more of the electrodes provides a detectable change in impedance. Since contact at any one or more of the electrodes provides a detectable change in impedance, impedance may be detected at any of the electrodes. For completeness, Applicants'

provide this technical distinction in comparison to Picard, Tchao and Lynes alone or in combination.

Claim 1 is not obvious over Picard or Tchao in view of Lynes. More specifically, Lynes "builds on a standard configuration of the ECIS system of Giaever and Keese." (Col. 9, ll. 55-57) In the standard ECIS system, "two electrodes are lithographed..." (Col. 3, ll. 45-55) and there are two electrodes, one being the sensing electrode and one being the counter electrode. Importantly, "In an ECIS system, the relative size of the sensing and counter electrodes can be significant. With large sensing electrodes, cell-related resistance signals become difficult to detect." (Col. 4, ll. 27-30) In addition, "When electrodes have a surface area of approximately 10^{-3} cm^2 or less, the impedance of the electrode-electrolyte interface at 4kHZ predominates, and in this situation, changes in resistance due to interaction of the cells with the electrode surface are clearly revealed." (Col. 4, ll. 32-37) "Due to the relative small size of the [sensing] electrode, resistance at the sensing electrodes predominates in the system." (Col. 3, ll. 63-65) In summary, the Lynes system operates by combining a small sensing electrode having a relatively small resistance with a large counter electrode "such that resistance at the sensing electrodes predominate." Thus, the device in Lynes operates by detecting changes at the sensing electrode. Given the teachings of Lynes, one skilled in the art would be limited to the sensing electrode – counter electrode system, whereas Applicants do utilize such a system. Therefore it would not be obvious for one skilled in the art to take the disclosure of Lynes and significantly alter it to provide a system where any of the electrodes can be used to detect changes and not solely the sensing electrode.

In addition to the significant difference in electrode design and operation, Lynes positions electrodes on substrate for horizontal migration, whereas Applicants' place electrodes on the porous membrane and Applicants' system is adapted for vertical migration. More specifically, Lynes discloses a radial diffusion system where a cell traverses a sensing electrode while horizontally migrating along the bottom of the substrate. In this system a chemoattractant radially diffuses through agarose positioned above the cell. Referring to Col. 10, ll. 21-30 of Lynes,

> "The cells loaded into the one or more cell containment volumes begin to move under the influence of the gradient established by diffusion of the chemoattractant species over

> the substrate **58** and under the agarose layer **64** in the direction of the gradient and interact with the one or more sensing electrodes **10** in their path. The cells eventually reach and move across the sensing electrode **10** located, in a preferred embodiment between the cell containment volume **68** and the chemical agent volumes **66** as illustrated in panel B of FIG. 2."

FIG. 2 of Lynes displays a sensing electrode **10** and a large counter electrode **40** placed on a substrate **58.** The sensing electrode is positioned horizontally between the cell containment volume **68** and chemical agent volume **66**. The agarose is positioned above the substrate for radial diffusion of the chemoattractant (across and parallel to the substrate). The cell traverses across the top of the sensing electrode when migrating along the bottom of the well or substrate. Lynes requires the use of a sensing electrode and a large countere electrode, the use of "chemical agent volumes" that are "interspersed among the array of one or more cell containment volumes" (Col. 5, ll. 14-16), the use of "biocompatible chemical gradient stabilizing medium" (Col. 5, ll. 25-27).

It is important to note that the "biocompatible chemical gradient stabilizing medium" is essential part of Lynes' system since Lynes clearly indicates that Boyden-type system having "microporous membrane" (Col. 2, ll. 39-40) on which the cells are placed has "a major limitation" that "the chemical gradient sensed by the cells is very steep and dissipates rapidly" (Col. 2, ll. 53-56). Lynes also indicates that the under-agarose assay in Lynes system "provides a significantly different type of cell environment that that utilized in a Boyden-type of assay" (Col. 3, ll. 28-30) and that "the resulting chemical gradient that is sensed by the cells in the cell containment volume comprises a greater volume and persists for a much longer time that the type of gradient that exists in the prior art Boyden-type chemotactic assay" (Col. 10, ll. 6-10). By pointing to flaws in previous Boyden systems, Lynes would be interpreted by those skilled in the art to pursue the detection of horizontal migration using sensing electrode – counter electrode configurations beneath a gradient. Thus objective evidence within Lynes itself supports the position that Applicants invention is not obvious. In addition, the use of "chemical agent volumes" and "biocompatible chemical gradient stabilizing medium" allows Lynes to position small sensing electrodes and large counter electrodes

in locations required by its inherent limitations. For example, it is required that "at least one of the sensing electrodes is between one cell containment volume and one chemical agent volume" (Col. 5, ll. 18-20). Again, Lynes is not able to place any electrode in such a location, but only the sensing electrode.

In contrast, Applicants have developed an electrode system that operates significantly different than Lynes and therefore Applicants are not limited to the confinements of the Lynes electrode system and its required placements. More specifically, Applicants' invention does not utilize a small sensing electrode and a larger counter electrode system as described in Lynes, does not utilize "chemical agent volumes" and does not utilize "biochemical gradient stabilizing medium". Applicants' invention includes at least two electrodes which have substantially same surface area and where cells contacting either electrode can result in an impedance change. In other words, all electrodes may be used to monitor impedance. This is provided by the element, "contact at any one or more of the electrodes provides a detectable change in impedance." In addition, the electrodes in Applicants' invention are disposed on the porous membrane and not a substrate as provided by Lynes. The result is that Applicants are not limited to Lynes' placement of electrodes and have advanced migration technology in an entirely different path. Structurally, Applicants' invention includes an upper chamber, a lower chamber and a biocompatible porous membrane with electrodes disposed thereon.

The structural distinctions between Applicants' electrodes and Lynes sensing-counter electrode system would result in significantly different methods of operation if Lynes were adapted to a migration or invasion test as developed by Applicants. More specifically, if Lynes were adapted to such an assay, impedance would only be measured when contact between a cell and the sensing electrode occurs because "resistance at the sensing electrodes predominates in the system"; whereas Applicants' invention detects changes in impedance when cells contact either of the two electrodes. Thus significant limitations found in Lynes' sensing-counter electrode system are not present in Applicants' technology. Applicants have therefore generated significant advances in the field of cell migration studies.

Moreover the positioning of the electrodes in Applicants' invention significantly deviates from the systems demonstrated by Lynes. In Applicants' invention electrodes are positioned on the biocompatible porous membrane, which allows cells to migrate therethrough. Lynes positions electrodes on a solid substrate beneath an agarose layer for horizontal migration. The systems are thus significantly different in design and operation.

Applicants agree with the Examiner that neither Picard nor Tchao disclose the use of electrodes in the detection of cells. Picard provides a multiwell insert device (e.g., 100a, 100b, 100c in FIG. 1B, 1D, 1F, 2, 3, 4, 5, 6, 7, 8 and 9) having two chambers with a membrane (e.g. 106 in FIG. 1B, 1D, 1F, 2, 3, 4, 5, 6, 7, 8 and 9) attached to the bottom of the upper chamber (e.g., 116 in FIG. 1B, 1D, 1F, 2, 3, 4, 5, 6, 7, 8 and 9) and having an incorporated sensor (e.g., 120 in FIG. 1B, 1D and 1F) for detecting an object passed through the membrane by measuring a change in a refractive index. Cells are optically detected on the surface of the lower chamber by an incorporated sensor for measuring a change in refractive index. More specifically referring to FIG. 1B, the cells **122** are detected at the bottom surface **112** of the lower well **108** with the sensor **120** measuring the refractive index. Referring to Page 2, Paragraph [0026], "The multiwell insert device includes a sensor **120** that detects in a label-free or independent manner an object 122 ...". Now referring to Page 2, Paragraph [0027], "The sensor **120** detects in a label-free or independent manner the presence of the object **122** in the lower chamber **116** by measuring a change in a refractive index caused by the presence of the object **122** on or near the surface **112**". Furthermore, referring to Page 3, Paragraph [0031], "Referring to FIG.3, there is a cross-sectional side view of the multiwell insert device **100**a which has incorporated thereon a grating-based planar waveguide sensor **120**a. Basically, the grating-based planar waveguide sensor **120**a is an optical biosensor which makes use of the refractive and coupling properties of light to detect the presence of cells **122**a on the surface **112** of the lower chamber **116**." Thus, the device disclosed in Picard includes a sensor for measuring refractive index. Multiple optical biosensors operated with different approaches are described in Picard "to detect the migrated cells", including "a grating-based planar waveguide sensor" in FIG. 3, " a grating-based planar waveguide sensor that utilizes an angular interrogation approach" in FIG. 4, "a grating-based planar waveguide

sensor that utilizes a spectral interrogation approach" in FIG. 5, "a grating-based surface plasma resonance (SPR) sensor" in FIG. 6 and "a prism-based surface plasma resonance (SPR) sensor" in FIG. 7 (See Page 1, Paragraphs [0014- 0018]). All these sensors are operated based on optical approaches for measuring refractive index. Migrated cells are detected in Picard only after they migrate from upper chamber to lower chamber through membrane and fall onto bottom "surface of lower chamber" to cause a change in refractive index (See Page 3, Paragraph [0031]; Page 3, Paragraph [0033-0035]; Page 4, Paragraph [0036]; FIG. 11).

Tchao is a two chamber system separated by a radiation opaque membrane. Cells labeled with a dye, such as a fluorescent dye, are placed in a first chamber and a chemoattractant in a second chamber. The labeled cells migrate through pores of the opaque membrane and are stimulated with electromagnetic radiation of one wavelength, and furthermore the radiation emitted by the stimulated cells is measured from the side of the opaque membrane closest to the second chamber. (see Col. 2, ll. 43-65) Referring to Col. 4, ll. 3-5, "As seen in FIG. 2, a space **28** is created between the radiation opaque membrane **10** and the bottom of the well **22**." Now referring to Col. 4, ll. 26-29, "With the preferred apparatus illustrated in FIGS. 1 and 2, this step would comprise stimulating and measuring the radiation from below the radiation opaque membrane, that is through space **28**". In addition, referring to Col. 4, ll 39-47, "The device **30** used to stimulate the cells and measure the emitted radiation will, of course, depend on the dye used to label and the type of apparatus used for the assay procedure. For example, if the plate apparatus of FIGS.**1** and **2** is used, a fluorescent plate reader, ..., can be used to advantage". Thus, the system disclosed in Tchao measures radiation (such as fluorescence) of labeled cells from a bottom second chamber with an optical-measurement device such as a plate reader. In Tchao's system, cells have to be labeled. Referring to Col. 5, ll. 8-10, "The only practical limitations on the cell type are its ability to exhibit a chemotactic response and its ability to be labeled".

The optical detection systems of Picard and Tchao are the essential part of their systems. For Picard, the system relies on an incorporated optical sensor for the measurement of refractive index based on optical approaches. Picard did not mention or address whatsoever the need of replacing the optical sensor with an impedance sensor.

For Tchao, the system replies on the use of optical-measurement device for measuring radiation from labeled cells that have migrated through the unique radiation-opaque membranes. Tchao did not mention or address whatsoever the need of replacing of the optical detection means with an impedance sensor. In fact, both Picard and Tchao rely on optical detection technologies. In view of the technologies provided by both Picard and Tchao, one skilled in the art would not find it obvious to significantly change direction in technological approaches to develop an electrode based system or even further to generate an electrode system where at least two electrodes are disposed on a porous membrane, having the same surface area and wherein contact at any one or more of the electrodes provides a detectable change in impedance.

Again referring back to Lynes, the use of ECIS electrode system having a small sensing electrode and a large counter electrode and the use of "chemical agent volumes" and "biochemical gradient stabilizing medium" are essential to Lynes' system. For Lynes, the system relies on the use of small sensing electrode and large counter electrode for detection of cells. The system also relies on the use of "chemical agent volumes" and "biochemical gradient stabilizing medium", without which the system cannot be formed. Lynes did not mention or address whatsoever the need of using a different structures. Indeed, Lynes criticizes Boyden-type system, which has "microporous membrane" (Col. 2, ll. 39-40), having "a major limitation" that "the chemical gradient sensed by the cells is very steep and dissipates rapidly" (Col. 2, ll. 53-56). Thus, clearly there is no motivation for Lynes to combine the small-sensing and large counter electrode structures with Boyden-type chambers (having microporous membrane separating two chambers) or even further to dispose electrodes on a porous membrane for detecting migration therethrough.

As discussed above, Lynes would be interpreted by those skilled in the art to pursue the detection of horizontal migration using sensing electrode – counter electrode configurations beneath a gradient. The objective evidence within Lynes itself supports the position that Applicants invention is not obvious. Also as discussed above, one skilled in the art would not find obvious to significantly change direction in technological approaches employed in Picard or Tchao from an optical detection system to an impedance-based system. Thus, Lynes would not be logically combined with Picard and

Tchao. Furthermore, at most the combination of Picard, Tchao and Lynes would require either a) the sensing (small) electrode / large counter electrode system of Lynes, b) the optical detection system of Picard with an incorporated optical sensor or c) the radiation detection system of Tchao with an optical-measurement device to detect the presence of cells. Applicants have significantly deviated from these technological approaches. In contrast Applicants' invention utilizes a system where at least two electrodes are disposed on a porous membrane, the at least two electrodes have substantially same surface area and contact at any one or more of the electrodes provides a detectable change in impedance. Thus the invention of claim 1 would not be obvious to one skilled in the art. Applicants respectfully request the rejections be withdrawn and claim 1 allowed.

Applicants have canceled claims 2 and 138. Claim 2 was canceled to economize on USPTO fees and the limitations of claim 138 have been incorporated into claim 1. Claims 6 and 8-11 depend from claim 1. Applicants incorporate by reference in their entirety the arguments set forth with respect to claim 1. The indications by the examiner with respect to claims 6 and 8-11 do not cure the deficiencies of a proper rejection of claim 1, thus claims 6 and 8-11 are not obvious over Picard, Tchao and Lynes. Applicants respectfully request the rejections of claims 6 and 8-11 be withdrawn and claims 6 and 8-11 allowed.

Follow up to Examiner Interviews and Responses to Examiner Summary

For completeness, Applicants address each recent Examiner interview. In the more recent Examiner interview on November 8, 2007 and in the follow up Examiner interview summary, the Examiner noted that comments with respect to the position of the electrodes on the membrane appeared to be persuasive in view of the prior art rejection of record. At the time of the interview, claim 24 included this limitation however claim 1 did not. Applicants have amended claim 1 to newly recite that at least two electrodes are disposed on the porous membrane.

In the previous Examiner interview on October 22, 2007 and in the follow up Examiner interview summary, the Examiner noted that if the limitation recited in claim 36 was incorporated into claim 1, then arguments should also address the references cited

against claim 36. For clarity, claim 36 (now canceled) previously included the limitation that at least two of the two or more electrodes have substantially the same surface area. Claim 1 has been amended to newly recite, migration of cells through the porous membrane permits contact between the migrating cells and one or more of the at least two electrodes of the lower chamber, wherein the at least two electrodes have substantially the same surface area, and further wherein the contact at any one or more of the at least two electrodes provides a detectable change in impedance.

The rejection of claim 36 relied on Picard, Tchao, Lynes in view of Ehret. Applicants incorporate herein the arguments set forth above against Picard, Tchao and Lynes with respect to claims 1, 6 and 8-11. For completeness, Applicants now address Ehret.

Ehret discloses an interdigitated electrode structure used to monitor impedance of cells grown in culture. Ehret fabricates electrodes on a solid substrate (wafer) as the bottom for a cell culture chamber and measures the impedance of cells grown in culture. Ehret does not provide a system for monitoring cell migration but instead a system for monitoring cell growth in culture. Thus, Ehret does not include an upper chamber, a lower chamber nor a biocompatible porous membrane that allows the passage of cells therethrough. In addition, Ehret does not disclose as system where at least two electrodes are disposed on the membrane for the detection of vertical migration of cells. Thus, if considered by one skilled in the art, the combination of Ehret must include the use of the Ehret electrode configuration with impedance-based migration devices as known in the art.

Ehret would not be logically combined with Picard and Tchao. The optical detection systems of Picard and Tchao are the essential part of their systems. Again, for Picard the system relies on an incorporated optical sensor for the measurement of refractive index based on optical approaches. Picard did not mention or address whatsoever the need of replacing the optical sensor with an impedance sensor. For Tchao, the system replies on the use of optical-measurement device for measuring radiation from labeled cells that have migrated through the unique radiation-opaque membranes. Tchao did not mention or address whatsoever the need of replacing of the optical detection means with an impedance sensor. In fact, both Picard and Tchao rely on

optical detection. In view of the technologies provided by both Picard and Tchao, one skilled in the art would not find obvious to significantly change direction of technological approaches to develop an electrode based measurement system.

With respect to Lynes, even if Ehret combined with Lynes provided sufficient information to one skilled in the art to lead to the replacement of the electrode system of Lynes with the electrode system of Ehret, the assay format as provided by Lynes would still be required. In other words even if Ehret permitted one skilled in the art to alter the Lynes electrode configuration, the format of the migration assay would still require detection of cell migration horizontally across a solid substrate with an agarose layer for radial diffusion of a chemoattractant positioned above the cell. It is important to note that the "biocompatible chemical gradient stabilizing medium" is essential part of Lynes' system since Lynes clearly indicates that Boyden-type system having "microporous membrane" (Col. 2, ll. 39-40) on which the cells are placed has "a major limitation" that "the chemical gradient sensed by the cells is very steep and dissipates rapidly" (Col. 2, ll. 53-56). Lynes also indicates that the under-agarose assay in Lynes system "provides a significantly different type of cell environment than that utilized in a Boyden-type of assay" (Col. 3, ll. 28-30) and that "the resulting chemical gradient that is sensed by the cells in the cell containment volume comprises a greater volume and persists for a much longer time that the type of gradient that exists in the prior art Boyden-type chemotactic assay" (Col. 10, ll. 6-10). By pointing to flaws in previous Boyden systems, Lynes would be interpreted by those skilled in the art to pursue the detection of horizontal migration using a sensing electrode-counter electrode configuration beneath a "chemical gradient". Thus, objective evidence within Lynes itself supports the position that Applicants' invention is not obvious. In addition, it is required that "at least one of the sensing electrodes is between one cell containment volume and one chemical agent volume" (Col. 5, ll. 18-20). Thus if the Ehret electrode configuration was combined with Lynes, the Ehret electrode system would merely replace Lynes and be positioned on a solid substrate and positioned beneath an agarose layer for use with radial diffusion assays.

In contrast, claim 1 includes an upper chamber, a lower chamber, and a biocompatible porous membrane with at least two electrodes disposed thereon. The

migration of cells through the porous membrane permits contact between the migrating cells and the one or more of the at least two electrodes of the lower chamber, wherein the at least two electrodes have substantially the same surface area and further wherein the contact at any one or more of the at least two electrodes provides a detectable change in impedance between or among the electrodes.

With respect to claims 6 and 8-11, these depend from claim 1. Applicants have canceled claims 2 and 138. Applicants incorporate by reference in their entirety the arguments set forth with respect to claim 1. Applicants respectfully request the rejections of claims 6 and 8-11 be withdrawn and claims 6 and 8-11 allowed.

2. Claims 24, 25, 28, 29, 143-145, 147 and 151-153

With respect to claims 24, 25, 28, 29, 143-145, 147 and 151-153, Applicants' incorporate the arguments set forth above with respect to a) Lynes requires a small sensing electrode and a large counter electrode system and "biochemical gradient stabilizing medium" and "chemical gradient volumes"; b) Picard requires an optical detection system with an incorporated optical sensor, and c) Tchao requires a radiation detection system with an optical-measurement device.

In contrast, Claim 24, from which claims 25, 28, 29, 143-145, 147 and 151-153 depend, recites, the device has two or more electrodes having substantially the same surface area fabricated on one side of a flexible biocompatible membrane that comprises at least one pore and the device has a surface suitable for cell attachment or growth, further wherein cell or attachment or growth results in cellular contact with at least one of the two or more electrodes further resulting in a detectable change in electrical impedance, resistance or capacitance.

As discussed above and incorporated herein, both Picard and Tchao rely on optical detection systems, which would not logically lead one skilled in the art to significantly change technological directions to develop an electrode system that includes at least two or more electrodes having the same surface area.

As discussed, Lynes' system relies on the use of "chemical agent volumes" and "biochemical gradient stabilizing medium" and "a small sensing electrode and a larger

counter electrode", and thus clearly one skilled in the art would not combine the small-sensing and large counter electrode structures of Lynes with Boyden-type chambers (having microporous membrane separating two chambers), much less a system as claimed by Applicants. Lynes' electrodes are fabricated on a plastic (non-porous) substrate. The electrodes in the Applicant's invention are fabricated on a porous flexible membrane. It takes a significant shift in scientific and technological approaches when electrodes are fabricated on solid substrates versus on porous, flexible membranes. In routine experimentation, one skilled in the art would not simply change the base material from solid substrate to a porous flexible membrane, since there are technical hurdles in fabricating electrodes on porous flexible membranes. This is supported by specification in paragraph [00120], which recites in part

> "Due to the "flexible" nature of thin membrane, special care may need to be taken in order to reproducibly and accurately make microelectrode structures on the membrane using photolithography methods. In particular, care should be taken to ensure that the membrane is stretched flat and made in good contact with the mask during the photo-masking process. In addition, as the membrane has at least one hole of appropriate size for cell migration/invasion, and if the hole or holes are present prior to electrode fabrication, care should be taken not to affect those holes during the fabrication process."

As provided, there are significant changes in production to encounter that require more than mere switching of surfaces. Thus, changing base material from solid substrates to a flexible porous membrane is not an obvious step.

Furthermore, as discussed above and incorporated herein, neither Lynes, Picard or Tchao demonstrate a system where two or more electrodes have substantially the same surface area fabricated on one side of a flexible biocompatible membrane comprising at least one pore and cellular contact with at least one of two or more electrodes results in a detectable change in electrical impedance, resistance or capacitance. Lynes requires contact with a sensing electrode because "Due to the relative small size of the [sensing] electrode, resistance at the sensing electrodes predominates in the system." (Lynes, Col. 3, ll. 63-65). Furthermore, Lynes' electrodes are not fabricated on a flexible biocompatible membrane comprising at least one pore. In addition, Lynes points out the

flaw in Borden chamber having "microporous membrane" and the objective evidence within Lynes itself supports the position that Applicants' invention is not obvious. Picard requires a detection of an optical property – refractive index by using an optical sensor and Tchao requires detection of radiation, such as fluorescence by using an optical-measurement device. Both Picard and Tchao rely on optical detection. In view of the technologies provided by both Picard and Tchao, one skilled in the art would not find obvious to significantly change direction of technological approaches to develop an electrode based measurement system or even further to generate an electrode system where at least two electrodes are fabricated on a biocompatible membrane that comprises at least one pore. Thus a combination of Lynes, Picard and Tchao would not render the invention of claim 24 obvious.

With respect to claim 25, Applicants provide a membrane that is between 5-50 microns thick. The thinness of this flexible membrane highlights the difficulties in applying a microelectrode array. Thin, flexible porous membranes tend to tear, can easily be deformed (for example, folded) and are not easily adaptable to technologies and systems that are used for producing electrodes on thick wafer substrates. In contrast Lynes applies a sensor electrode – counter electrode configuration on a non-porous, solid plastic substrate. Thus, one skilled in the art would not find it obvious, given Lynes' disclosure, to fabricate electrodes having substantially the same surface area on a thin flexible porous membrane.

With respect to claims 28, 29, 143-145, 147 and 151-153, each depend from claim 24. Applicants incorporate by reference in their entirety the arguments set forth with respect to claim 24. The indications by the examiner with respect to claims 25, 28, 29, 143-145, 147 and 151-153 do not cure the deficiencies in the rejection of claim 24, thus claims 25, 28, 29, 143-145, 147 and 151-153 are not obvious over Picard, Tchao and Lynes. Applicants respectfully request the rejections of claims 25, 28, 29, 143-145, 147 and 151-153 be withdrawn and claims 25, 28, 29, 143-145, 147 and 151-153 allowed.

Applicants respectfully request the rejections of claims 1, 6, 8-11, 24, 25, 28, 29, 49, 143-145, 147 and 151-153 be withdrawn and all claims allowed.

Follow up to Examiner Interview and Response to Examiner Summary

In the Examiner interview on October 22, 2007 and in the follow up Examiner interview summary, the Examiner noted that if the limitation recited in claim 36 was incorporated into claim 24, then arguments should address the references provided in claim 36. A rejection of claim 24 as applied through 36 is discussed in section C below.

B. Claims 9, 24, 25, 28 and 29, are not obvious over Picard (US 2004/0091397) or Tchao (US 5,601,997) in view of Lynes et al (US 6,723,523) taken further in view of Springer et al (US 5,514,555)

The Examiner has rejected claims 9, 24, 25, 28 and 29 under 35 U.S.C. §103(a) as allegedly being obvious over Picard or Tchao in view of Lynes in further view of Springer.

Applicants' Response

The combination of references of Picard, Tchao and Lynes has been discussed above and is incorporated by reference herein in their entirety. Springer detects the migration of cells by staining using techniques such as antibody conjugated to a fluorphore.

With respect to claim 9, this is dependant from claim 1. Claim 1 was amended to recite that at least two electrodes are disposed on the biocompatible porous membrane, the at least two electrodes have substantially the same surface area and contact at any one or more of the electrodes provides a detectable change in impedance. Springer does not recite or provide guidance for these elements and thus does not provide the deficiencies identified above in the combination of references of Picard, Tchao or Lynes that would permit one skilled in the art to find Applicants' invention obvious. Thus Applicants respectfully request this rejection be withdrawn.

With respect to claim 24, Applicants have amended claim 24 to recite the device at least two electrodes have substantially the same surface area fabricated on one side of a flexible biocompatible membrane that comprises at least one pore and has a surface

suitable for cell attachment or growth, further wherein cell or attachment or growth results in cellular contact with at least one of the two or more electrodes further resulting in a detectable change in electrical impedance, resistance or capacitance. Springer does not disclose a system where at least two electrodes have substantially the same surface area fabricated on one side of a flexible biocompatible membrane that comprises at least one pore and cellular contact with at least one of two or more electrodes results in a detectable change in electrical impedance, resistance or capacitance. Since Springer does not cure these deficiencies with respect to Picard, Lynes and Tchao, claim 24 is not obvious over the Picard, Lynes, Tchao and Springer.

With respect to claim 25, Applicants have amended claim 25 to include the width of the membrane is from 5-50 microns. This is not demonstrated in Springer, Picard, Lynes or Tchao. Thus, claim 25 is not obvious over Springer, Picard, Lynes and Tchao.

With respect to claims 28 and 29, these claims depend from claim 24. Thus, since Springer does not cure the deficiencies in the arguments with respect to the combination of references of Picard, Lynes and Tchao as provided above, claims 25, 28 and 29 are not obvious over Picard, Lynes, Tchao and Springer.

C. Claims 36, 43, 50, 51, 62, 65, 68 and 69 are not obvious over Picard (US 2004/0091397) or Tchao (US 5,601,997) in view of Lynes et al (US 6,723,523) taken further in view of Ehret et al (Biosensors)

Claims 36, 43, 50, 51, 62, 65, 68 and 69 were rejected under 35 USC §103(a) as allegedly being obvious over Picard or Tchao in view of Lynes et al and further in view of Ehret et al.

Applicants' Response

Applicants have canceled claim 36. Applicants have amended claim 43 to depend from claim 24.

With respect to claim 43, Applicants have amended claim 43 to depend from claim 24 and herein incorporate the arguments set forth above with respect to the combination of references of Picard, Tchao and Lynes that the combination of references

do not demonstrate Applicants' requirement that the device comprises at least two electrodes having substantially the same surface area fabricated on one side of a flexible biocompatible membrane that comprises at least one pore and has a surface suitable for cell attachment or growth, further wherein cell or attachment or growth results in cellular contact with at least one of the two or more electrodes further resulting in a detectable change in electrical impedance, resistance or capacitance and thus can not be relied upon to maintain a proper obviousness rejection.

Ehret does not cure the deficiencies in combination of references of Picard, Tchao and Lynes to find Applicants invention obvious. More specifically the references alone or in combination do not suggest to one skilled in the art to provide a device including a two or more electrodes fabricated one side of a flexible biocompatible membrane that includes at least one pore, wherein the device has a surface suitable for cell attachment or growth, further wherein cell attachment or growth results in cellular contact with at least one of the two or more electrodes further resulting in a detectable change in electrical impedance, resistance or capacitance.

As discussed above, Lynes positions electrodes on substrate for horizontal migration, whereas applicants' system is adapted for vertical migration. As a review, Lynes discloses a radial diffusion system where a cell traverses a sensing electrode while horizontally migrating along the bottom of the substrate. In this system a chemoattractant radially diffuses through agarose positioned above the cell. Referring to Col. 10, ll. 21-30 of Lynes,

> "The cells loaded into the one or more cell containment volumes begin to move under the influence of the gradient established by diffusion of the chemoattractant species over the substrate **58** and under the agarose layer **64** in the direction of the gradient and interact with the one or more sensing electrodes **10** in their path. The cells eventually reach and move across the sensing electrode **10** located, in a preferred embodiment between the cell containment volume **68** and the chemical agent volumes **66** as illustrated in panel B of FIG. 2."

FIG. 2 of Lynes displays a sensing electrode **10** and a large counter electrode **40** placed on a substrate **58.** The sensing electrode is positioned between the cell

containment volume **68** and chemical agent volume **66**. The agarose is positioned above the substrate for radial diffusion of the chemoattractant (across and parallel to the substrate). The cell traverses across the top of the sensing electrode when migrating along the bottom of the well or substrate. Lynes requires the use of a sensing electrode and a large countere electrode, the use of "chemical agent volumes" that are "interspersed among the array of one or more cell containment volumes" (Col. 5, ll. 14-16), the use of "biocompatible chemical gradient stabilizing medium" (Col. 5, ll. 25-27).

It is important to note that the "biocompatible chemical gradient stabilizing medium" is essential part of Lynes' system since Lynes clearly indicates that Boyden-type system having "microporous membrane" (Col. 2, ll. 39-40) on which the cells are placed has "a major limitation" that "the chemical gradient sensed by the cells is very steep and dissipates rapidly" (Col. 2, ll. 53-56). Lynes also indicates that the under-agarose assay in Lynes system "provides a significantly different type of cell environment than that utilized in a Boyden-type of assay" (Col. 3, ll. 28-30) and that "the resulting chemical gradient that is sensed by the cells in the cell containment volume comprises a greater volume and persists for a much longer time that the type of gradient that exists in the prior art Boyden-type chemotactic assay" (Col. 10, ll. 6-10). By pointing to flaws in previous Boyden systems, Lynes would be interpreted by those skilled in the art to pursue the detection of horizontal migration using a sensing electrode-counter electrode configuration beneath a "chemical gradient". Thus, objective evidence within Lynes itself supports the position that Applicants' invention is not obvious. In addition, the use of "chemical agent volumes" and "biocompatible chemical gradient stabilizing medium" allows Lynes to position small sensing electrodes and large counter electrodes in locations required by its inherent limitations. For example, it is required that "at least one of the sensing electrodes is between one cell containment volume and one chemical agent volume" (Col. 5, ll. 18-20).

In contrast, the present invention does not utilize a small sensing electrode and a large counter electrode system as described in Lynes, does not utilize "chemical agent volumes" and does not utilize "biochemical gradient stabilizing medium". Applicant's invention includes two or more electrodes having substantially the same surface area fabricated on a flexible biocompatible membrane that includes at least one pore, wherein

the device has a surface suitable for cell attachment or growth and cell attachment or growth results in cellular contact with at least one of the two or more electrodes further resulting in a detectable change in electrical impedance, resistance or capacitance.

Picard provides a multiwell insert device (e.g., 100a, 100b, 100c in FIG. 1B, 1D, 1F, 2, 3, 4, 5, 6, 7, 8 and 9) having two chambers with a membrane (e.g. 106 in FIG. 1B, 1D, 1F, 2, 3, 4, 5, 6, 7, 8 and 9) attached to the bottom of the upper chamber (e.g., 116 in FIG. 1B, 1D, 1F, 2, 3, 4, 5, 6, 7, 8 and 9) and having an incorporated sensor (e.g., 120 in FIG. 1B, 1D and 1F) for detecting an object passed through the membrane by measuring a change in a refractive index. Cells are optically detected on the surface of the lower chamber by an incorporated sensor for measuring a change in refractive index. More specifically referring to FIG. 1B, the cells **122** are detected at the bottom surface **112** of the lower well **108** with the sensor **120** measuring the refractive index. Referring to Page 2, Paragraph [0026], "The multiwell insert device includes a sensor **120** that detects in a label-free or independent manner an object 122 ...". Now referring to Page 2, Paragraph [0027], "The sensor **120** detects in a label-free or independent manner the presence of the object **122** in the lower chamber **116** by measuring a change in a refractive index caused by the presence of the object **122** on or near the surface **112**". Furthermore, referring to Page 3, Paragraph [0031], "Referring to FIG.3, there is a cross-sectional side view of the multiwell insert device **100**a which has incorporated thereon a grating-based planar waveguide sensor **120**a. Basically, the grating-based planar waveguide sensor **120**a is an optical biosensor which makes use of the refractive and coupling properties of light to detect the presence of cells **122**a on the surface **112** of the lower chamber **116**." Thus, the device disclosed in Picard includes a sensor for measuring refractive index. Multiple optical biosensors operated with different approaches are described in Picard "to detect the migrated cells", including "a grating-based planar waveguide sensor" in FIG. 3, " a grating-based planar waveguide sensor that utilizes an angular interrogation approach" in FIG. 4, "a grating-based planar waveguide sensor that utilizes a spectral interrogation approach" in FIG. 5, "a grating-based surface plasma resonance (SPR) sensor" in FIG. 6 and "a prism-based surface plasma resonance (SPR) sensor" in FIG. 7 (See Page 1, Paragraphs [0014- 0018] of Picard). All these sensors are operated based on optical approaches for measuring refractive index. Migrated cells are detected in Picard only

after they migrate from upper chamber to lower chamber through membrane and fall onto bottom "surface of lower chamber" to cause a change in refractive index (See Page 3, Paragraph [0031]; Page 3, Paragraph [0033-0035]; Page 4, Paragraph [0036]; FIG. 11 of Picard).

Tchao is a two chamber system separated by a radiation opaque membrane. Cells labeled with a dye, such as a fluorescent dye, are placed in a first chamber and a chemoattractant in a second chamber. The labeled cells migrate through pores of the opaque membrane and are stimulated with electromagnetic radiation of one wavelength, and furthermore the radiation emitted by the stimulated cells is measured from the side of the opaque membrane closest to the second chamber. (see Col. 2, ll. 43-65) Referring to Col. 4, ll. 3-5, "As seen in FIG. 2, a space **28** is created between the radiation opaque membrane **10** and the bottom of the well **22**." Now referring to Col. 4, ll. 26-29, "With the preferred apparatus illustrated in FIGS. 1 and 2, this step would comprise stimulating and measuring the radiation from below the radiation opaque membrane, that is through space **28**". In addition, referring to Col. 4, ll 39-47, "The device **30** used to stimulate the cells and measure the emitted radiation will, of course, depend on the dye used to label and the type of apparatus used for the assay procedure. For example, if the plate apparatus of FIGS.**1** and **2** is used, a fluorescent plate reader, ..., can be used to advantage". Thus, the system disclosed in Tchao measures radiation (such as fluorescence) of labeled cells from a bottom second chamber with an optical-measurement device such as a plate reader. In Tchao's system, cells have to be labeled. Refer to Col. 5, ll. 8-10 of Tchao, "The only practical limitations on the cell type are its ability to exhibit a chemotactic response and its ability to be labeled".

The optical detection systems of Picard and Tchao are the essential part of their systems. For Picard, the system relies on an incorporated optical sensor for the measurement of refractive index based on optical approaches. Picard did not mention or address whatsoever the need of replacing the optical sensor with an impedance sensor. For Tchao, the system replies on the use of optical-measurement device for measuring radiation from labeled cells that have migrated through the unique radiation-opaque membranes. Tchao did not mention or address whatsoever the need of replacing of the optical detection means with an impedance sensor. In fact, both Picard and Tchao rely on

optical detection. In view of the technologies provided by both Picard and Tchao, one skilled in the art would not find obvious to significantly change direction of technological approaches to develop an electrode based measurement system or even further to generate an electrode system where at least two electrodes are fabricated on a flexible biocompatible membrane that comprises at least one pore and has a surface suitable for cell attachment or growth and wherein contact at any one of more of the electrodes provides a detectable change in impedance.

Furthermore, when the device of Applicants' invention in Claim 24 is used for monitoring cell migration/invasion, Applicants' invention provides significant advances in monitoring cell migration in comparison with Picard and Tchao. Comparing with Picard where migrated cells can be detected only after they fall onto the bottom surface of the lower chamber (as discussed above), the present invention allows for direct monitoring of cell migration. As soon as the migrated cells attach to and are in contact with any one electrode fabricated on the flexible, porous biocompatible membrane of the device in Claim 24, their presence would contribute to a detectable change in impedance and these cells would be monitored. To be detected, migrated cells in the present invention do not need to travel the distance between the membrane and the bottom surface of the lower chamber. Thus, measurement of cell migration using device of Applicants' invention with electrodes fabricated on a flexible, porous membrane is more direct and more rapid than that in Picard system. Comparing with Tchao system where migrated cells have to be pre-labeled with a dye, Applicants' invention when used for measurement of cell migration does not need "label cells", reducing needed assay time and assay cost and improving assay performance. Indeed, the limitation of Tchao system was the same as those of described in Paragraph [0009] of Applicant's application, where a discussion of a BD Biosciences' FluoroBlok PET membrane system was presented. Refer to Paragraph [0009] of Applicants' application, which recites in part,

> "The system developed in BD Bioscineces uses a light-tight FluoroBlok PET membrane that is specifically designed to block the transmission of light .... The testing cells should be first stained with a fluorescence dye and ..... Using this assay system the invasion assay productivity and throughput are significantly improved. However, since not every cell type can be homogenously labeled with the

> fluorescent dye and labeling has in some cases been found to alter cell invasion and migration, the application of the system has also been significantly limited."

Thus, Applicants' invention provides significant advances to cell migration assay devices.

Now referring to Ehret, Ehret discloses an interdigitated electrode structure used to monitor impedance of cells grown in culture. Ehret fabricates electrodes on a solid substrate (wafer) as the bottom for a cell culture chamber. Ehret does not fabricate electrodes on a flexible biocompatible membrane having at least one pore but instead fabricates electrodes on a solid, non-porous substrate (wafer) as the bottom for a cell culture chamber and measures the impedance of cells grown in culture. Thus, the Ehret system is not adapted for use with a porous flexible membrane system but instead a cell culture system that utilizes a rigid or solid substrate. Ehret did not mention or address whatsoever the need of changing base material from solid substrates to a porous flexible membrane. One skilled in the art would likely agree that porous flexible membranes may not be suited for use as the bottom for a cell culture chamber. Thus, any adaptation of Ehret electrode structures to a porous membrane system would far exceed routine optimization but instead requires a significant shift in scientific and technological approach. In addition, in routine experimentation, one skilled in the art would not simply change the base material from solid substrate to a porous flexible membrane, since there are technical hurdles in fabricating electrodes on porous flexible membranes. This is supported by specification in paragraph [00120], which recites in part

> "Due to the "flexible" nature of thin membrane, special care may need to be taken in order to reproducibly and accurately make microelectrode structures on the membrane using photolithography methods. In particular, care should be taken to ensure that the membrane is stretched flat and made in good contact with the mask during the photo-masking process. In addition, as the membrane has at least one hole of appropriate size for cell migration/invasion, and if the hole or holes are present prior to electrode fabrication, care should be taken not to affect those holes during the fabrication process."

Thus, changing base material from solid substrates to porous membrane for fabricating electrodes is a significant shift in scientific and technological approach and is not an obvious step.

Furthermore, as discussed above and incorporated herein, neither Lynes, Picard , Tchao, nor Ehret demonstrates a system where two or more electrodes are fabricated on one side of a flexible biocompatible membrane comprising at least one pore and where a surface is suitable for cell attachment or growth, further wherein cell or attachment or growth results in cellular contact with at least one of the two or more electrodes further resulting in a detectable change in electrical impedance, resistance or capacitance. Lynes requires contact with a sensing electrode because "Due to the relative small size of the [sensing] electrode, resistance at the sensing electrodes predominates in the system." (Lynes, Col. 3, ll. 63-65). Furthermore, Lynes' electrodes are not fabricated on a flexible biocompatible membrane comprising at least one pore. Lynes points out the flaw in Borden chamber having "microporous membrane" and the objective evidence within Lynes itself supports the position that Applicants' invention is not obvious. Picard requires a detection of an optical property – refractive index by using an optical sensor and Tchao requires detection of radiation, such as fluorescence by using an optical-measurement device. Both Picard and Tchao rely on optical detection. In view of the technologies provided by both Picard and Tchao, one skilled in the art would not find obvious to significantly change direction of technological approaches to develop an electrode based measurement system or even further to generate an electrode system where at least two electrodes are fabricated on a flexible biocompatible membrane that comprises at least one pore. As discussed above, Ehret does not fabricate electrodes on a flexible biocompatible membrane having at least one pore but instead fabricates electrodes on a solid, non-porous substrate (wafer). Changing base material from solid substrates to porous membrane for fabricating electrodes is a significant shift in scientific and technological approach and is not an obvious step. Importantly, as discussed above, Applicants' invention provides significant advances to cell migration assay devices. Thus a combination of references of Lynes, Picard, Tchao and Ehret would not render the invention of claim 24 obvious.

Since Ehret does not cure these deficiencies with respect to Picard, Lynes and Tchao as required in claim 24, claim 43 is not obvious over the Picard, Lynes, Tchao and Ehret. Thus claim 43, which depends from claim 24 is not obvious over a combination of references of Picard, Lynes, Tchao and Ehret.

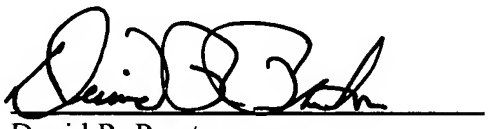
With respect to claims 50, 51, 62, 65, 68 and 69, these claims depend from claim 24. Since Picard, Lynes, Tchao and Ehret alone or in combination do not render claim 24 obvious as provided above, these references alone or in combination can not render claims 50, 51, 62, 65, 68 and 69 obvious.

III. Conclusion

Applicants respectfully request all rejections be withdrawn and request an allowance be granted for the present application.

Respectfully submitted,

Date: Dec. 13, 2007



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